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## Repositioning chloroquine as antiviral prophylaxis against COVID-19: potential and challenges

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The Coronavirus Disease 2019 (COVID-19) pandemic is advancing globally, and pharmaceutical prophylaxis is one solution. Here, we propose repositioning chloroquine (CQ) as prophylaxis against COVID-19. CQ blocks viral attachment and entry to host cells and demonstrates efficacy against a variety of viruses, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19. Furthermore, CQ is safe, inexpensive, and available. Here, we review the antiviral mechanisms of CQ, its in vitro activity against coronaviruses, its pharmacokinetics (PK) and adverse effects, and why it could be more efficacious as a prophylactic rather than as a therapeutic, given the infection dynamics of SARS-CoV-2. We propose two prophylactic regimens based on efficacy and risk considerations. Although it is largely preclinical data that suggest the potential of CQ, properly planned prophylactic trials and further research are urgently needed.

## Introduction

Since its reported outbreak in late 2019 [1], COVID-19 has exploded from a few people with a respiratory disease in the Chinese city of Wuhan to a pandemic of millions of cases. Current methods of pandemic control are largely confined to public (travel restrictions, quarantines, avoidance of gatherings, and school closures) and personal (face mask use and hand hygiene) health measures, whereas vaccine development will cost billions of dollars and might be as far as 18 months away from deployment [2].

Pharmaceutical antivirals are not only potentially therapeutic, but have also been successfully applied pre and post exposure as prophylaxis against viral infections, such as influenza [3] HIV [4], cytomegalovirus [5], and respiratory syncytial virus [6]. Using influenza as a model for preventive management of respiratory viral pandemics, the key concerns are surges in community attack rates and healthcare system demand [7], which in turn lead to disruptions in healthcare, with potentially disastrous social and economic ramifications. In their systemic review and meta-analysis of effective interventions to contain an influenza pandemic, Saunders-Hastings et al. identified vaccination and antiviral prophylaxis as two major pharmaceutical interventions that can be effective [8]. However, to date, neither have we developed a vaccine nor is there any approved or established antiviral prophylaxis in deployment against COVID-19.

In the case of COVID-19, hiding in plain view is a plausible and potential prophylaxis option that can be achievable by repositioning the old drug CQ. CQ was developed as chemoprophylaxis against malaria and has known immunomodulatory and antiviral properties. Although largely overtaken by newer and more effective agents, CQ is a drug that has been in use for over half a century against malaria [9] and still one of the most prescribed drugs in the world [10].

Here, we review relevant experimental results of CQ as an antiviral as well as its pharmacokinetics (PK) and toxicities to suggest CQ as a potential candidate drug that could be repositioned as an antiviral prophylactic against COVID-19.

## Methodology

Two researchers independently searched the electronic databases MEDLINE, Scopus, and EMBASE, as well as preprint servers, including bioRvix, medRvix, and Preprints.org, for relevant and pertinent articles, reviews, protocols, and preprints for this review. Three subtopics were separately searched: (i) CQ or its derivative hydroxychloroquine (HCQ) as antivirals, especially against HCoVs, including SARS-CoV-2, the causative agent of COVID-19; (ii) CQ and HCQ toxicities and complications, especially with reference to COVID-19; and (iii) CQ and HCQ dosing, PK and pharmacodynamics (PD). Search strategies combining relevant medical subject headings (MeSH) and keywords for the three subtopics above were ('chloroquine' OR 'hydroxychloroquine') AND, respectively, (i) ('virus' OR 'antiviral' OR 'viral' OR 'SARS-CoV' OR 'SARS-CoV-2' OR 'coronavirus' OR 'COVID-19' OR 'HCoV'; (ii) ('toxicity' OR 'complication' OR 'risk' OR 'cardiac' OR 'arrhythmia' OR 'overdose'); and (iii) ('dose' OR 'dosage' OR 'dosing' OR 'pharmacokinetic' OR 'pharmacodynamics' OR 'pharmacology'). The above searches were further supplemented with text-book and monograph articles on the background on the development of CQ and HCQ, their conventional use, as well as pharmacology. News reports on CQ and HCQ experimental use and toxicities relating to COVID-19 and their regulatory updates were identified via searches using keywords 'chloroquine' OR 'hydroxychloroquine' AND 'COVID-19' OR 'SARS-Co-V-2' OR 'coronavirus' OR 'FDA' in English via Google News (https://news.google.com) in the date range February 1, 2020 to June 1, 2020. Furthermore, relevant clinical trials, systematic reviews, meta-analyses, and bibliographic references from the above-identified studies were further manually screened to identify additional studies. The searches were independently conducted by both authors without restrictions in English with the date of search ranging from November 23, 1963 (the beginning of the MeSH indexing) to March 1, 2020, and updated on June 2, 2020.

## Background to CQ

CQ is a 4-aminoquinoline that is most well known as an antimalarial. It was originally synthesized in 1934 and its full clinical development involved investigators from six countries on five continents over a decade before clinical trials confirmed its therapeutic value as an antimalarial drug [11]. HCQ is a hydroxylated derivative of CQ that was introduced in 1945. CQ was clinically introduced as a prophylactic treatment for malaria in 1947 and subsequently included by the WHO in its model list of essential medicines, which includes drugs deemed essential in addressing public health needs globally. In the USA, CQ is approved for the treatment and prophylaxis of uncomplicated malaria where CQ-sensitive malaria is present, and for the treatment of extraintestinal amebiasis. Besides their anti-malarial properties, CQ and HCQ also have established immunomodulatory and anti-inflammatory effects [12] and current nonapproved or repositioned use of these drugs includes the potential treatment of a spectrum of diseases, both non-infectious and infectious, such as a range of cancers [13], rheumatoid arthritis, systemic lupus erythematosus (SLE), systemic sclerosis, primary progressive multiple sclerosis, Q fever, Whipple's disease, and a variety of fungal and viral infections [14].

## CQ as an antiviral: in vitro and in vivo studies

The bioactivity of CQ against viruses was first reported 50 years ago [15], and its potential to be repositioned as a broad-spectrum antimicrobial against bacteria, fungal, and viral infections was proposed over a decade ago [16].

CQ has direct and indirect antiviral effects. Direct antiviral activity of CQ has been identified against a range of 30 viruses, mostly by in vitro studies [16]. Its mechanisms of direct inhibition by impeding viral entry as well as disrupting postentry viral envelope maturation have been reviewed elsewhere [17]. It was subsequently demonstrated that CQ targeting of endosomal acidification and resultant alkalinization of cellular organelles and inactivation of pH-dependent enzymatic processes impede both viral entry and replication, and is the basis of its potential as an antiviral [18].

Upon attachment to cells, a virus needs to fuse to the host cell to deliver the viral genome. Preventing viral entry by inhibiting attachment and fusion are ideal for prophylaxis against infection. This approach has been successful with HIV and has been demonstrated to be viable in vitro with CQ against the Ebola (EBOV), influenza, and Marburg viruses [19].

Another direct antiviral mechanism of CQ involves impairment of pH-dependent protease and glycosyltransferase enzymes in the endoplasmic network needed for postentry viral envelope maturation, which has been demonstrated in experiments with Flaviviruses [20], dengue (DENV), and chikungunya (CHIKV) viruses.

Besides acting directly on the virus, there are possible indirect antiviral effects that impede viral cellular entry and infection. For example, CQ was demonstrated to interfere with terminal glycosylation of the cellular receptor angiotensin-converting enzyme 2 (ACE2), which facilitates entry of SARS-CoV, thus potentially reducing virus–receptor binding and abrogating infections; separate cell culture studies with SARS-CoV demonstrated the effectiveness of CQ in preventing infection if the drug is added 24 h before infection and even if added 5 h post infection [21]. Significantly, SARS-CoV-2 is also an animal-derived HCoV in the same Sabrecovirus subgenus of the Coronaviridae virus family as SARS-CoV, and shares the same ACE2 pathway to initiate an infection [22]. Besides SARS-CoV, CQ also demonstrated antiviral activity against five out of seven known human corona viruses, including SARS-CoV-2 [23], MERS-CoV [24], HCoV-229E [25], and HCoV-OC43 [26]. In the case of SARS-CoV-2 as in SARS-CoV, time-of-addition assays demonstrated that CQ functioned at both entry and postentry stages of infection in the Vero E6 cell assay used [23].

Specifically, Wang et al. reported the 50% effective concentration (EC50) of CQ against SARS-CoV-2 using infected Vero E6 cells as determined by CCK8 assay to be 1.13 $\mu$M, with $EC_{90}$ 6.90 $\mu$M, indicating potent viral inhibition at micromolar concentrations achievable with conventional clinical CQ dosing [23]. For comparison with activity against other HCoVs, the $EC_50$ was 3.6 $\mu$M for MERS-CoV [24], between 2.3 $\mu$M and 4.4 $\mu$M for SARS-CoV [21], and 0.3 $\mu$M for HCoV-OC43 replication in HRT-18 cells [26].

In animals, CQ can prevent DENV infection in Aotus monkeys [27], reduce Zika virus-induced mortality when administered soon after infection [28], protect mice against a deadly challenge dose of EBOV [29], and reduce mortality of lethal HCoV-OC43 infection in newborn C57BL/6 mice when CQ was acquired through the placenta or via maternal milk [26].

## CQ as an antiviral: clinical studies

There have only been a few small clinical studies using CQ or its derivative HCQ as antivirals to date. In HIV, HCQ at 800 mg daily for 8 weeks was found to effect a 0.6 $\log_{10}$ reduction of HIV-1 load ($P = 0.022$) compared with untreated controls [30]. In a chronic active hepatitis B study, alanine aminotransferase was normalized in patients who received 50–450 mg of CQ for a median of 12 months [31]. Another trial investigating the antiviral effects of CQ for 3 days beginning 72 h after infection by DENV and demonstrated CQ reduction of occurrence of

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DENV hemorrhagic fever as well as decrease in patients' pain intensity and improved activity performance [32].

As for CQ or HCQ studies in COVID-19, several clinical studies using CQ or HCQ have already reported results and >30 ongoing therapeutic trials have been registered globally since February 2020 [33]. Some encouraging early results include a narrative Chinese interim report on an ongoing multicenter controlled trial involving ten hospitals, finding improved lung imaging and viral conversion as well as less pneumonia exacerbation and reduced clinical disease duration compared with controls in >100 patients with COVID-19 treated with 500 mg of CQ twice daily for 10 days [34]. Separately, a French group reported the efficacy of HCQ in 26 patients with COVID-19, where 200 mg three times a day reduced viral carriage on Day 6 post treatment compared with controls [35], whereas another study of 80 patients with COVID-19 by the same group found that HCQ treatment with azithromycin resulted in a rapid decline in the viral load, with a seroconversion rate of 83% on Day 7 and 93% on Day 8 [36]. However, a more recent systematic review of seven CQ and HCQ trials in COVID-19, including the Chinese interim report and the two French studies above, concluded that the trials reporting so far were poorly designed with various degrees of bias, such that there is yet insufficient evidence to establish the efficacy of either drug [37].

Separately, there are currently planned enrollments of tens of thousands of patients globally to test CQ and HCQ as prophylaxis against COVID-19, including a well-planned Oxford University-sponsored 40 000 subject randomized double-blind study in the health-care setting using CQ and HCQ to be carried out in Asia, Europe, and Africa (NCT 04303507) and a 3000-subject postexposure pre-emptive therapy trial launched in North America (NCT 04308668).

## CQ: PK and PD considerations

CQ is rapidly and well absorbed orally with good bioavailability (>75%) and peak serum levels are achieved within 2–3 h. Approximately 55% of the drug in the plasma is bound to nondiffusible plasma constituents. It undergoes primarily hepatic metabolism by cytochrome P450 enzymes and has a long plasma terminal elimination half-life of 1–2 months; after a single dose, the drug can be found in the liver and urine for up to 5 years. The long half-life reflects its high volume of distribution (>100 l/kg), which extends into aqueous compartments, with approximately 50% of the metabolites undergoing renal clearance [38]. Significantly for

its potential use against a respiratory virus, a peak tissue:plasma concentration ratio >300 is obtained in many tissues, including lungs, and the concentration increased with chronic administration at 10 mg/kg/week in a rodent study [39].

To successfully reposition CQ as an antiviral prophylactic against a respiratory virus such as COVID-19, we need to formulate an optimal dosing regimen that can achieve relevant viral inhibition in respiratory tissues with a reasonable margin of safety. Fortunately, because CQ has long been in use, we have extensive pharmacological data on the drug, including for children [40], during pregnancy [41], for short-term prophylaxis against malaria, as well as for long-term administration in autoimmune disease [42].

## CQ COVID-19 prophylaxis: proposed dose regimens

Current clinical dosing recommendations for CQ depend on the indication. For malaria, the WHO currently recommends an adult dose of 500 mg (base) weekly for prophylaxis, and 25 mg/kg over 3 days for treatment for acute attack in uncomplicated cases [43]. In autoimmune diseases, the generally advocated dose is 250–500 mg daily for rheumatoid arthritis [44] and 250 mg per day in SLE [45].

Dose finding for a repurposed drug should be guided by effective drug levels against the target condition, as well as informed by dose ranges and known toxicities applied and reported from the existing approved or indicated usage of the drug. Dosage can also be guided by animal models, because CQ PK in mice is similar to those reported for humans [29]; rodent studies can also provide useful guidance for effective dosing in higher animals.

The established safe clinical application of CQ ranges from the dosing of 500 mg weekly in malaria prophylaxis to 500 mg daily or more for acute malaria or chronic autoimmune conditions [46]. These same dose ranges appear adequate to exert antiviral effects on HCoVs, such as SARS-CoV [21] and SARS-CoV-2 based on *in vitro* results (see earlier).

The weekly CQ dose of 500 mg for malaria prophylaxis yields only 0.9–1.3 $\mu$M in whole blood the day after treatment and troughs at 0.4–0.5 $\mu$M before the next dose [47]; this is below the $EC_{50}$ for inhibition of COVID-19 and, thus, not optimal for COVID-19 prevention.

However, the low end of the dose range of CQ used for the treatment of rheumatoid arthritis (3.6 mg/kg or 250 mg a day) generates plasma CQ concentrations of 1–1.6 $\mu$M [42], which

would be in range of the $EC_{50}$ for SARS-CoV-2 inhibition [23]. Separately, a higher but shorter dose of CQ for acute malaria at 8 mg/kg/day for 3 days achieves a serum concentration of 9 $\mu$M [48], which is above the $EC_{90}$ value of 6.90 $\mu$M against SARS-CoV-2, and can be adopted for postexposure prophylaxis.

Based on the above analysis and synthesis, we propose two prophylactic schedules for CQ antiviral against COVID-19: (i) CQ 8 mg/kg/day for 3 days in postexposure but asymptomatic cases, ideally to be taken within hours after known viral exposure based on *in vitro* data that CQ might be significantly effective even 5 h after virus adsorption and infection [21]; and (ii) CQ 500 mg a day as chronic prophylaxis for individuals in the midst of a local outbreak or in endemic areas or work settings, such as medical facilities, with a high risk of exposure; to reduce to 250 mg a day after 30 days and to continue until the threat of infection is abated.

The higher initial dose of 500 mg for chronic prophylaxis is based on achievable serum levels in the same range [42] of the $EC_{50}$ and $EC_{90}$ range of 1.13–6.90 $\mu$M against the virus [23] and because we expect higher tissue concentrations in respiratory tissue than in the serum. The reduced dose of 250 mg after 30 days of treatment is proposed again based on large increased and cumulative concentrations in lungs and other organ tissues after repeated dosing over time [39], as well as a concern for long-term toxicity after prolonged use (see later).

## CQ toxicity and cardiac risk in COVID-19

CQ is generally considered safe and well tolerated, with its adverse effects well delineated. For relevance, we limit our review largely to adverse effects and potential toxicities related to the two dose regimens proposed earlier for COVID-19 prophylaxis.

Our first proposed regimen, of CQ 8 mg/kg/day for 3 days in postexposure but asymptomatic cases, is similar to the treatment dose for acute malaria attack. The potential adverse effects for this short duration regimen include nausea, anorexia, abdominal pain, vomiting, dizziness, headache, blurry vision, and pruritus [49]. A small Phase I trial found these adverse effects to be dose related and generally <15% except for headache, which was the most common adverse effect at 21%, with doses comparable or slightly above those of this regimen [50]. These adverse effects are usually mild, transient, and can be minimized by taking CQ with food.

Our second proposed regimen is a prophylactic dose for those at high risk of acquiring the

infection and is at 250–500 mg daily for the duration of susceptibility, which could last months but is unlikely to be years. This dosing schedule is consistent with dosages used in autoimmune disorders, although not for as long. A relevant review of CQ toxicities related to chronic use at 250–500 mg daily for SLE included 95 articles published between 1982 and 2007 and confirmed the general clinical experience that toxicity is infrequent, mild, and usually reversible [51].

Besides the earlier-mentioned minor adverse effects, chronic administration of CQ leads to tissue accumulation and poses a unique and rare set of toxicities, including retinal, ocular, and neurological damage. Retinal toxicity is a particularly serious concern in chronic use because of its debility. According to one report, the incidence of toxic ocular effects was <1% of adults treated, such as CQ at 4 mg/kg/day (~250 mg daily) for 5 years or less, but increases with duration of treatment [52].

Of specific concern to our proposed use in COVID-19 is the issue of potential cardiac toxicity of CQ and HCQ reported in patients with COVID-19. A systematic review of the literature on cardiac complications attributed to CQ and HCQ conducted before the emergence of COVID-19 involved 86 articles reporting on 127 patients using CQ (58.3%) or HCQ (39.4%) or both. The review found that most reported cases with cardiac complications had been treated for a long time (median 7 years) with a high cumulative dose (median 803 g for CQ and 1235 g for HCQ) and conduction disorders were the main complication (85% of cases) [53]. Although cardiac complications from CQ and HCQ are historically rare for short-term use, the safety of these drugs needs to be completely reassessed in the context of COVID-10, which is a new target disease with different comorbidities in separate populations; this is particular relevant given that it is already known that cardiovascular disease might be the most common comorbidity for COVID-19, cardiac complications are common [54], and where most currently investigated drugs for the condition are already thought to present heightened cardiac risks [55]. Indeed, a systematic review of the arrhythmogenic potential of short-term CQ and HCQ in nine studies reporting on 1491 patients with COVID-19 found that ~10% of patients developed QT prolongation to a degree that generally led to drug withdrawal (QTc ≥500 ms or change >60 ms) [56], that two out of 37 patients in a study who were treated with a high dose of CQ at 2 g daily developed ventricular arrhythmia [57], and a

first-degree atrioventricular block occurred in one patient in a separate study [58]. Thus, these studies suggest overall that CQ and HCQ can frequently induce significant QTc interval prolongation and increase the risk of arrhythmia, especially at higher doses.

Overall, decades-long experience with the acute and chronic use up to years of various doses of CQ show a relatively low incidence of adverse effects. Traditionally, the main concern in long-term administration is retinopathy and other tissue toxicities associated with drug accumulation, which one does not expect in short-term prophylaxis; nevertheless, the cardiac risk for COVID-19 is separate concern that needs to be addressed.

Given that the target population for COVID-19 prophylaxis is expected to be healthier compared with patients hospitalized with COVID-19 being treated with CQ or HCQ and the proposed prophylactic doses are lower than treatment doses, we realistically expect lower cardiac risks than reported in treatment trials when the drugs are used for prophylaxis. However, a hypothetically lower cardiac risk will need to be confirmed by ongoing prophylactic trials. Meanwhile, we would recommend avoiding the use of these drugs in those with a cardiac history or who are concurrently taking another arrhythmogenic drug. Furthermore, a careful consideration of risks *versus* benefits, a thorough cardiovascular history taking and exam, and a screening electrocardiogram, especially of older patients or those with a cardiac history, before initiating prophylactic CQ would be prudent.

## Discussion

Current inadequacies in containing the COVID-19 pandemic are evidenced by the prolonged social lockdown in areas succumbing to the pandemic and continued increase of cases despite public containment efforts and personal preventative measures by citizens worldwide. As infections soar, healthcare systems have been taxed to the brink and fear and panic have escalated. Pharmaceutical efforts involve the rapid development of effective vaccines as well as discovery of novel therapeutics against the virus, but these efforts are costly and take time [59]. Drug repositioning, where existing drugs on the market with established safety profiles are redeployed for a new indication, can lead to less costly and faster approval and deployment [60]; such an approach should be especially considered when there is an urgent and timely need for effective therapeutics, as in the current pandemic.

## Antiviral prophylaxis in viral epidemics

Four major pharmacological prophylaxis to prevent and protect populations during a viral pandemic are vaccination [61], passive neutralizing antibodies [62], convalescent plasma [63], and small-molecule drugs [29]. There is active research on vaccine development as well as the use of neutralizing antibodies and convalescent plasma for COVID-19, but currently no prophylactic agent is ready to enter the clinic [64].

Small-molecule drugs as therapeutics against novel viruses have the advantage of stability and convenience of oral administration. Two development paths could be *de novo* synthesis of inhibitors targeting unique viral proteins involved in its infection process or screening existing drug databases for potential drug candidates [29]. These approaches have been deployed for other HCoVs with pandemic potential, such as SARS-CoV [65] and MERS-CoV [66], and are underway for SARS-CoV-2; however, deployment of these measures is only established for influenza.

Conceptually, massive antiviral prophylaxis might be effective in containing a viral pandemic, as the use of neuraminidase inhibitors against influenza demonstrates [8]. A Cochrane Collaboration review found this to reduce the risk of developing influenza, and multiple randomized studies confirmed its utility irrespective or pre- or postexposure use, offering 67–89% protection in individuals and households [67], thus potentially setting a model for drug prophylaxis for COVID-19.

## Drug repositioning against viruses and COVID-19

Given clinical experience of use and the fact that human safety studies have already been conducted, repositioned drugs offer many advantages as a path of least resistance for large-scale public deployment, especially in the midst of a rapidly advancing viral pandemic. Drug development risk, time, and cost are dramatically reduced because the drug candidates would have established safety and PK profiles, while chemical optimization, toxicology, bulk manufacturing, as well as formulation development have already been addressed [68].

There is a long history of drug repositioning for viral diseases, and there are currently ~24 drugs and drug combination candidates for this purpose, targeting Zika, HCoVs, influenza, herpes, norovirus, rotavirus, and EBOV, some of which are already in Phase II/III trials [69].

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Specifically, strong cases have already been made to reposition existing drugs, including CQ and HCQ, against HCoVs such as SARS-CoV [70] and MERS-CoV [24], and several repositioned drugs, including CQ and HCQ, are currently in trials for both the treatment and prevention of COVID-19.

## Distinction of CQ as treatment versus prophylaxis for COVID-19

CQ has been previously called upon as a therapeutic agent against HCoVs (MERS-CoV, SARS-CoV, and now SARS-CoV-2)|, but the initial emphasis as well as clinical reports has been on the treatment of hospitalized cases. During February 2020, based on encouraging preliminary findings from ongoing clinical trials in China, a Chinese Government-sponsored conference accepted the findings of the activity of CQ against COVID-19 and recommended the drug for inclusion in the next version of the *Guidelines for the Prevention, Diagnosis, and Treatment of Pneumonia Caused by COVID-19* issued by China's National Health Commission [34]. In March 2020, the US Food & Drug Administration also authorized the temporary use of CQ or HCQ in patients hospitalized with COVID-19, where clinical trials are not available or participation is not feasible [71], despite equivocal results of reported clinical trials on the efficacy of CQ so far and some concern about its cardiac safety.

However, we need to distinguish between treatment of patients hospitalized with COVID-19 *versus* prevention of SARS-CoV-2 infection. Differences in viral load and dynamics at inception of antiviral treatment might translate into significant differences in efficacy, and different characteristics and comorbidities in target populations impute different risk profiles for CQ toxicities.

Clinical outcomes are known to be correlated to the timing of antiviral treatment initiation in general, as demonstrated in the case of influenza [72]. Japanese researchers mathematically modeling SARS-CoV-2 viral dynamics in hosts using viral load data from 38 patients and then performing *in silico* experiments to evaluate antiviral drug response demonstrated that antiviral treatment is unlikely to be effective if initiated much after symptom onset. When using duration of viral shedding as an endpoint, the researchers also found early initiation of treatment significantly reduced viral shedding, whereas late initiation did not affect its duration [73]. Indeed, a small observational study on HCQ with azithromycin postulated that early administration of the antivirals might be what

accounted for their 100% success rate in their treatment of 19 COVID-19 pneumonia cases [74].

The target population for CQ prophylaxis of COVID-19, such as front-line healthcare workers, is also likely to be younger with fewer comorbidities compared with patients hospitalized with COVID-19, translating to significantly lower cardiac risk, although the hypothetical enhancement in efficacy and lowered risk need to be properly assessed and confirmed *via* well-designed clinical trials.

## Limitations, obstacles, and further research

Although there is convincing laboratory data that CQ inhibits SARS-CoV-2 at clinically relevant dosages, such preclinical efficacy might not translate into the clinic. As an example, studies reported inhibitory effects of CQ *in vitro* against viruses such as influenza [75] but it was not effective as a prophylactic *in vivo* [76] and a clinical trial did not demonstrate preventative efficacy [77]. Also, although CQ has demonstrated inhibition of CHIKV in a dose-dependent manner *in vitro* [78], a trial on French Reunion during a CHIKV outbreak did not demonstrate clinical benefit [79]. Thus, it is possible that that CQ might yet be ineffective against SARS-CoV-2 despite promising *in vitro* efficacy.

Indeed, we currently lack an animal model to test CQ against SARS-CoV-2 [80], and reported clinical trials so far on CQ and HCQ as treatment for COVID-19 are equivocal because of small sample sizes and poor designs, despite a few encouraging reports [37]. Furthermore, major prophylactic trials are underway and have not yet been reported.

From the research angle of COVID-19 prophylaxis, there is urgent need for private and public funding into basic science as well as clinical research into the efficacy of CQ. This would include studies on its effect on viral dynamics to identify optimal timing and dosing for prophylaxis, finding and testing an animal model for prophylactic efficacy, with further PD studies on important issues, such as CQ tissue concentration over repeat dosing, to guide future trial designs, and further studies into the mechanisms of CQ cardiac toxicity as well as the development of diagnostic protocols or tools to identify those at risk. Finally, the development and validation of related compounds or derivatives, such as single enantiomers of CQ, which might be more efficacious and/or less toxic [81], as well as further clinical trials of CQ or HCQ in combination with other potentially synergistic antivirals or

adjuvants, all deserve urgent and concerted attention.

## Concluding remarks

CQ has significant advantages as a candidate for antiviral prophylaxis in the current COVID-19 pandemic, where no current vaccine or antiviral prophylaxis is in place. Its demonstrated mechanisms of action of preventing viral entry and fusion, evidence of *in vitro* efficacy at clinically achievable doses, high tissue concentration, as well as some preliminary clinical evidence of efficacy as treatment all support its potential preventative role. Its safety record and low cost at the doses we propose also imply a favorable benefit or cost: risk ratio if proven effective for prophylaxis. Therefore, we should adhere to the official advisories that CQ or HCQ only be used under strict monitoring as part of national emergency programs or in clinical trials against COVID-19, while agencies, institutions, and governments must spare no effort and expense in the funding, design, deployment, and reporting of clinical trials to assess seriously this potential solution for COVID-19.

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